TABLE 1
Coxsackieviruses, Group A*

Virus Type	Strain
A1	Tompkins
A2	Fleetwood
A3	Olson
A4	High Point
A5	Swartz
A6	Gdula
A7	Parker
A8	Donovan
A9	Bozek
A10	Kowalik
A11	Belgium-1
A12	Texas-12
A13	Flores
A14	G-14
A15	G-9
A16	G-10
A17	G-12
A18	G-13
A19	NIH-8663
A20	IH-35
A21	Kuykendall or Coe
A22	Chulman
A23	Joseph

*Type 23 was found to be identical with echovirus 9. Echovirus type 34 is related to coxsackievirus A24 as a prime strain.

NOTE: All reference products must be tested in the user's laboratory before, or when, they are used to evaluate the test products. If all reference products perform as described, they may be used to evaluate the products.

PROCEDURAL OUTLINE

1. **Test Procedure.** Serial twofold or fourfold dilutions of serum, heated at 56 C 30 min, are titrated against the virus dilution containing 2 X 100 LD_{50}. The mixture is allowed to incubate at 37 C for 1 h and inoculated 0.03 ml i.p. into less than 1-day-old suckling mice, 1 litter for each dilution.
2. **Stability of the Final Reaction.** The serum Nt test in mice should be examined for 14 days, at least twice daily for the first 3 days, and once daily for the remainder of the time.
3. **Reading of Results.** Recordings of each litter should be made at each observation using the standardized abbreviations for missing, eaten, dead, paralyzed, etc.
4. **Quality Control**
   a. The virus back titrations, included in each test, should demonstrate 32 to 320 LD_{50} (1.5 to 2.5 logs) of virus.
   b. Serum controls: equal volumes of initial serum dilution and Hanks' BSS should not demonstrate any evidence of toxicity or infection in the mice.
   c. In heterologous testing, the dilution of reference antiserum containing 20 neutralizing units should completely protect its homologous virus against 100 LD_{50} virus dilution.
   d. Each reference antiserum should neutralize its respective homologous virus at a dilution previously determined or as indicated on the vial.
   e. Uninoculated mice and mice inoculated with diluent should not demonstrate any evidence of toxicity or infection.

Materials Required But Not Provided

1. Stock virus cultures of all coxsackievirus group A types.
2. Reference animal antisera to all coxsackievirus group B types, with their respective stock virus cultures, optional.
3. Reference animal antisera to cross-reacting echovirus types (9 and 34) and their respective virus cultures, optional.
4. Suckling mice, less than 1-day-old, 1 litter for each serum-virus dilution, each virus dilution in the back titration, serum control, and uninoculated controls.
5. Hanks' Balanced Salt Solution (BSS).
6. The serum Nt procedure, including lists of all additional reagents, equipment, and glassware needed, is described in detail in the brochure *Serum Neutralization Test for the Evaluation of Viral Antisera* (G05), which is available upon request from:

   Centers for Disease Control
   Center for Infectious Diseases
   Attn: Office of Scientific Services
   Biological Products Branch
   Atlanta, Georgia 30333

INTERPRETATION OF RESULTS

1. **Virus Titration.** The end point of the virus titration is the highest logarithmic dilution, calculated by the Reed and Muench method, which kills or infects 50% of the inoculated mice.[3]
2. **Serum Neutralization.** The end point of the Nt test is the highest dilution, calculated by the Reed and Muench method, which protects 50% of the mice against infection when the optimal 100 LD_{50} units of virus is used.[3]
3. **Product Specification**

   Satisfactory products should demonstrate the following performance characteristics:

   a. **Potency:** The minimum acceptable homotypic titer is 1:512.

   b. **Specificity:** Heteotypic titers to other group A coxsackieviruses listed in Table 1 must be no greater than one-thirty-second (1/32) of the homotypic titer. If published data indicates that this is unavoidable, heterotypic titer of one-eighth (1/8) the homotypic is allowable.[3] Heteologous titers to group B coxsackieviruses and to those echoviruses which replicate in mice will be no greater than one-thirty-second (1/32) the homologous titer.

4. **Limitations of the Procedure.** The Nt test is limited by the care with which it must be performed, read, and the results interpreted. Maximum sensitivity and specificity of a test will not be achieved unless the laboratorian carefully (1) calculates dilutions and measures volumes, (2) observes and records reactions in animals, and (3) determines the end points.

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COXSACKIEVIRUS GROUP A REFERENCE NEUTRALIZATION REAGENTS

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COXSACKIEVIRUS GROUP A REFERENCE NEUTRALIZATION REAGENTS

INTENDED USE

Coxsackievirus group A types 1 through 24 animal antisera are to be used as reference in the serum neutralization (Nt) test to determine the performance characteristics of similar reagents from other sources.

SUMMARY AND EXPLANATION OF THE TEST

The Nt test performed in suckling mice is the method of choice in the specific identification of the group A coxsackievirus. If the results of the Nt test with this group of viruses are to be comparable when different lots of reagents are used, the reagents must meet minimum potency and specificity requirements. The products distributed with this brochure meet the requirements of the Centers for Disease Control (CDC) Specifications and are for use in evaluating products from other sources.[1]

PRINCIPLES OF THE PROCEDURE

The Nt test is based on the capacity of specific antibodies in serum to neutralize the infectivity of the homologous viral agent.

REAGENTS

Material Provided

Coxsackievirus group A types 1 to 24 Reference Animal Antisera for Neutralization.

COXSACKIEVIRUS GROUP A REFERENCE ANIMAL ANTISERA are prepared in rabbits inoculated with the strains listed in Table 1.[2] The antisera are dispensed in 2 ml volumes and freeze-dried. When titrated in suckling mice against 100 LD_{50} doses of the homotypic strain, the sera have titers $\geq$ 1:512. Heterotypic titers to other group A types (Table 1) do not exceed one-thirty-second (1/32) of the homotypic titer except where this is recognized.[1,3] Cross-relationships exist between coxsackieviruses A3 and A8, A11 and A15, A13 and A18. Heterologous reactivity to Group B coxsackieviruses and to echoviruses should not exceed one-thirty-second (1/32) the homologous titer. No preservative is added. The products are *For Reference In Vitro Diagnostic Use*. Sera rehydrated with distilled water and stored at -20 C are stable for 1 year.

BIBLIOGRAPHY

1. Diagnostic Products Evaluation Branch, 1974. Specifications and Evaluation Methods for Immunological and Microbiological Reagents. Vol. II. Chlamydial, Mycoplasmal, Rickettsial, and Viral. 4th ed., DHEW, PHS, CDC, Atlanta, GA. 30333
2. Chappell, W. A., L. A. White, and W. C. Gamble. 1984. Production Manual for Viral, Rickettsial, Chlamydial, and Mycoplasmal Reagents, 6th ed., DHHS, PHS, CDC, Atlanta, GA. 30333
3. Melnick, J. L., H. A. Wenner, and C. A. Phillips. 1979. Enteroviruses *In* Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. E. H. Lennettee and N.J. Schmidt, eds. 5th ed., Amer. Pub. Hlth. Assoc., Washington, D.C. p. 471-534.

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